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**Genotype dependent responses to levels of sibling  
competition over maternal resources in mice**

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**Abstract:** Research on phenotypic plasticity has often focused on how a given genotype responds to changing physical environments such as temperature or diet. However, for many species the social environment plays an equally important role due to competition for resources. During early development, the level of competition for limited (maternally provided) resources will often depend critically on the number of siblings. Therefore, competition among siblings should drive the evolution of genes that allow flexible responses to realized levels of competition and maternal resource availability. However, it is unknown whether genetically based differences between individuals exist in their response to the social environment that affects their future development. Using a quantitative trait locus approach in an experimental population of mice we demonstrate that effects of sibling number on body weight depend on individual genotype at seven loci, over and above the general negative litter size effect. Overall, these litter size-by-genotype interactions considerably modified the degree to which increasing litter size caused reduced weight. For example at one locus this effect leads to a 7% difference in body weight at week 7 between individuals experiencing the extremes of the normal range of litter sizes in our population (five to nine litter mates). The observed interaction between genotype and the competitive environment can produce differences in body weight that are similar in magnitude to the main effect of litter size on weight. Our results show that different genotypes respond to the social environment differentially and that interaction effects of genotype with litter size can be as important as genotype-independent effects of litter size.

46

## 47    **Introduction**

48            Phenotypic plasticity describes the ability of organisms to respond to changes  
49    in the environment (West-Eberhard 2003) and is generally referred to as flexibility  
50    when phenotypic change is reversible and as developmental plasticity when it is not  
51    (Stearns 1989; Piersma and Drent 2003). Plasticity may be adaptive if the range of  
52    phenotypes shown by a given genotype across environments, or the reaction norm,  
53    increases fitness when compared to a single phenotype in these environments (Via &  
54    Lande 1985; DeWitt *et al.*, 1998). Given the obvious fitness advantages of plasticity,  
55    studies sought to elucidate associated costs and constraints as plasticity has not been  
56    as universally observed as one might have expected (Snell-Rood *et al.*, 2010). A large  
57    part of this research focused on how a given genotype responds to changes in the  
58    physical environment such as diet or temperature. Yet, for many species the social  
59    environment is certainly of equal importance in determining individual fitness and  
60    explaining trait variation. How do given genotypes respond to changing social  
61    environments? Further, research into costs and constraints of plasticity requires an  
62    understanding of the underlying genetics as pleiotropy is an important source of  
63    evolutionary constraint, and because genetic variation is the prerequisite for evolution  
64    (e.g. Scheiner 1993; DeWitt *et al.*, 1998; Auld *et al.*, 2010). In this study, we tackle  
65    these issues and investigate whether genetic variants can respond differentially to a  
66    changing social environment in an experimental population of mice by focusing on  
67    effects of the competitive environment on body weight.

68            An adaptive response to changing levels of competition may be favoured by  
69    natural selection as the associated cost / benefit trade-off will change as well (e.g.  
70    Stockley and Parker 2002; Wright and Leonhard 2002). This seems particularly  
71    relevant to early development because levels of competition may be indicative of

72 available resources during this crucial developmental period (Gyekis *et al.*, 2011).  
73 Sibling competition over access to resources is common in species that provide  
74 significant parental care and have multiple offspring in a litter or brood (Mock and  
75 Parker 1997). In mammals with multiple offspring per litter, sibling competition is  
76 manifested largely in scramble competition, rather than contest competition, and is  
77 thus crucially dependent on the number of competitors (MacNair and Parker 1979;  
78 Mock and Parker 1997; Hager and Johnstone 2005). In mice the number of litter  
79 mates is rarely greater than the number of nipples (10), but access is often limited to  
80 one side of the female and offspring remain staunchly attached until all milk supply is  
81 depleted (Gilbert 1995). Moreover, teats differ in their productivity (Barnard *et al.*,  
82 1998), exacerbating sibling competition over the most productive nipples (usually the  
83 anterior ones). Females have been shown to increase milk supply as litter size  
84 increases (Knight *et al.*, 1986) which may mitigate competition on average but, at the  
85 same time, per capita milk supply may decrease and thus competition will increase.

86         The number of siblings at birth may serve as an indicator of expected postnatal  
87 sibling competition for maternal resources, and the number of litter mates at weaning  
88 may be indicative of post-weaning competition for resources. In rodents, the number  
89 of competitors may decrease or increase before weaning because pups may die or fall  
90 victim to prey, or another female may produce a litter in the same nest (several  
91 females often litter together in mice, König 1997) and pups are nursed by either  
92 female (König 1994). Consequently, levels of competition and associated costs  
93 (Trivers 1974; Clutton-Brock 1991; Godfray 1991) can increase or decrease  
94 postnatally. The key question we address here is whether any response to this change  
95 in the competitive environment has a genetic basis that differs between individuals or  
96 populations.

97           Our aim for this study was to investigate whether bodyweight and growth  
98 during the first 10 weeks of life is affected by the number of litter mates at birth or at  
99 weaning and whether these effects depend on individual genotype. Body weight is a  
100 key indicator of resource utilization during development and often a good predictor of  
101 fitness under natural conditions where weight is associated with higher reproductive  
102 potential, advantages in intra-sexual competition and female mate choice. Using a  
103 quantitative trait locus (QTL) design, we first investigate main effects of litter size,  
104 independent of genotype, and then establish whether individual genotype interacts  
105 with the number of litter mates at birth or at weaning. This enables us to investigate  
106 genetic variation in the response to the competitive environment, manifested in  
107 differential responses to the number of competitors for different genotypes. We  
108 predict that genotypes show different responses to the postnatal competitive  
109 environment.

110

## 111   **Methods**

112   Our study population is an intercross of two inbred mouse strains that were selected  
113 for divergent bodyweight at day 60, the Large (LG/J) and Small (SM/J) strains  
114 (Goodale 1938; MacArthur 1944). The two strains differ in litter size: while *Large* has  
115 an average litter size of 6.1, *Small* has an average litter size of 5.0, a difference of  
116 18%; Ehrich *et al.*, 2003). For our analysis we used the F<sub>2</sub> and F<sub>3</sub> generation that  
117 originated from the matings of ten Large males to ten Small females resulting in 52 F<sub>1</sub>  
118 individuals. These F<sub>1</sub> individuals were randomly mated to generate 510 F<sub>2</sub> mice,  
119 which, after random mating, produced 1632 individuals of the F<sub>3</sub> generation in 200  
120 full-sib families. At birth half litters were cross-fostered in 158 families (Kramer *et*  
121 *al.*, 1998). Thus, the size of cross-fostered litters may be larger or smaller compared

122 with litter size at birth. It is important to bear in mind that such differences in litter  
123 size can cause effects from as early as week 1 body weight and not just after weaning.  
124 In this analysis we focus on body weights taken once weekly from week 1 to week 10  
125 as well as pre-weaning (week 1-3) and postweaning growth (week 3-10) as  
126 phenotypes. We have previously analyzed these traits for main effects considering the  
127 autocorrelation between the weights, splitting the growth period in two, early and late,  
128 (Hager *et al.*, 2009b). A genome scan using such a multivariate approach will pick up  
129 QTL that affect growth in this particular period. However, a disadvantage of this  
130 approach is that loci that affect only few traits will be missed and given the pleiotropic  
131 nature of most QTL does not yield a picture of when during development QTL begin  
132 to show their effect, when they decrease and stop. We have thus analyzed the above  
133 traits separately, following Wolf *et al.*, (2008).

134 All F<sub>2</sub> and F<sub>3</sub> mice were genotyped at 353 polymorphic single nucleotide  
135 polymorphic markers (SNPs) that were evenly spaced (4-5 cM apart) across the  
136 genome, except where the two strains are monomorphic, using the Illumina Golden  
137 Gate assay (Wolf *et al.*, 2008). Haplotypes were reconstructed in Pedphase using the  
138 Integer Linear Programming (ILP) algorithm (Li and Jiang 2005) to produce a set of  
139 unordered haplotypes for the F<sub>2</sub> generation and a set of ordered (by allelic parent-of-  
140 origin) haplotypes for the F<sub>3</sub>. We distinguish four ordered genotypes denoted *LL*, *LS*,  
141 *SL*, *SS*, (paternal / maternal allele) with the *L* allele originating from the LG/J strain  
142 and the *S* allele from the SM/J strain.

143 We first analysed the effects of two litter size parameters on growth and  
144 development: litter size at birth (*LSB*; i.e. size of the litter born by the dam) and litter  
145 size at weaning (*LSW*). Using the Mixed Procedure in SAS (SAS version 9.1.3; SAS  
146 Institute, Cary, NC, USA) we fitted a mixed model using maximum likelihood to

147 model the trait as a function of two litter size parameters with biological and foster  
 148 family (*dam*, *nurse*) as random effect class variables to control for shared  
 149 environmental effects. Our aim was to establish which litter size parameter, if any,  
 150 affected weekly weights and growth.

151 We then used the marker loci to scan the genome for quantitative trait loci  
 152 (QTL) that interacted with either litter size at birth or with litter size at weaning to  
 153 affect weekly weights and growth. In a first step we assigned the four ordered  
 154 genotypes at the marker loci additive (*a*), dominance (*d*), and parent-of-origin (*i*)  
 155 genotypic index scores following Wolf *et al.*, (2008). These index scores are arrayed  
 156 in a genetic design matrix to relate variation in the mean phenotypes (i.e. genotypic  
 157 values) of each of the ordered genotypes ( $\overline{LL}, \overline{LS}, \overline{SL}, \overline{SS}$ ) to a vector of genetic  
 158 effects:

159

$$160 \begin{bmatrix} \overline{LL} \\ \overline{LS} \\ \overline{SL} \\ \overline{SS} \end{bmatrix} = \begin{bmatrix} 1 & 1 & 0 & 0 \\ 1 & 0 & 1 & 1 \\ 1 & 0 & 1 & -1 \\ 1 & -1 & 0 & 0 \end{bmatrix} \begin{bmatrix} r \\ a \\ d \\ i \end{bmatrix}$$

161

162 This linear equation can be used to solve for the genetic effects by inverting the  
 163 design matrix and multiplying it by the vector of genotypic values to yield a definition  
 164 of the genetic effects (in terms of genotypic values):

165

$$166 \begin{bmatrix} r \\ a \\ d \\ i \end{bmatrix} = \begin{bmatrix} \frac{\overline{LL}}{2} + \frac{\overline{SS}}{2} \\ \frac{\overline{LL}}{2} - \frac{\overline{SS}}{2} \\ \frac{\overline{LS}}{2} + \frac{\overline{SL}}{2} - \frac{\overline{LL}}{2} - \frac{\overline{SS}}{2} \\ \frac{\overline{LS}}{2} - \frac{\overline{SL}}{2} \end{bmatrix}$$

167



168 where  $r$  is the reference point for the model (the mid-point between homozygotes), the  
 169 additive effect is defined as half the difference in the mean phenotype of the two  
 170 homozygotes, the dominance effect is the difference of the mean heterozygote  
 171 phenotype from the mean of the homozygotes, and the genomic imprinting effect is  
 172 half the difference in mean phenotype between the two reciprocal heterozygotes  
 173 (Wolf *et al.*, 2008).

174 The genotypic index scores for a locus were used in a linear mixed model  
 175 fitted by maximum likelihood using the Mixed Procedure in SAS. In the first of these  
 176 models (Model 1) we included the three genetic effects ( $a$ ,  $d$ ,  $i$ ) and the two litter size  
 177 parameters ( $LSB$  and  $LSW$ ) as main effects, and the six pair-wise interactions between  
 178 the genetic and litter size parameters. The biological and foster family ( $dam$ ,  $nurse$ )  
 179 were included as random effect class variables to control for the background  
 180 influences of other loci and shared environmental effects that can inflate significance  
 181 values. Cross-fostering was not included in this model as we have previously shown  
 182 that there is no main effect of cross-fostering in this data set (Hager *et al.*, 2009a). The  
 183 fixed effects in Model (1) can be expressed as a linear model where  $Y_j$  is the trait  
 184 value (ten weekly weights or growth) of individual  $j$  and  $X_{a(j)}$ ,  $X_{d(j)}$ ,  $X_{i(j)}$  are the  
 185 genotypic index scores for the direct genetic effects (additive, dominance and  
 186 imprinting) of individual  $j$ :

$$\begin{aligned}
 188 \quad Y_{(j)} = & LSW_{(j)} + LSB_{(j)} + aX_{a(j)} + dX_{d(j)} + iX_{i(j)} + LSW_{(j)} * aX_{a(j)} + LSW_{(j)} * dX_{d(j)} + \\
 189 \quad & LSW_{(j)} * iX_{i(j)} + LSB_{(j)} * aX_{a(j)} + LSB_{(j)} * dX_{d(j)} + LSB_{(j)} * iX_{i(j)} + e_{(j)} \quad (1)
 \end{aligned}$$

190  
 191 To generate a test for the overall effect of a locus we generated a likelihood ratio test  
 192 by subtracting the  $-2 \log$  likelihood from Model 1 generated by the Mixed Procedure

193 from the  $-2$  log likelihood from a reduced model that included only  $LSB$ ,  $LSW$ , and  
 194 the same random effects as in Model (1). This difference in the  $-2$  log likelihoods of  
 195 the two models (reduced model minus full model, which always gives a positive  
 196 value) is approximately  $\chi^2$  distributed with nine degrees of freedom (i.e. the two  
 197 models differ by a total of nine model terms). The probability values calculated from  
 198 the  $\chi^2$  distribution (with 9 d.f.) were then transformed into a log probability ratio  
 199 (LPR) in order to make them comparable to LOD scores ( $LPR = -\log_{10}[\text{probability}]$ ).

200 To distinguish between interactions of genotype with litter size at birth or at  
 201 weaning we fitted two further models with the same random effects as in Model (1),  
 202 the three main genetic effects, the two litter size parameters and either the interactions  
 203 of genotype and litter size at birth (2) or genotype and litter size at weaning (3):

204

$$205 \quad Y_{(j)} = LSW_{(j)} + LSB_{(j)} + aX_{a(j)} + dX_{d(j)} + iX_{i(j)} + LSB_{(j)} * aX_{a(j)} + LSB_{(j)} * dX_{d(j)} + LSB_{(j)} \\ 206 \quad * iX_{i(j)} + e_{(j)} \quad (2)$$

207

$$208 \quad Y_{(j)} = LSW_{(j)} + LSB_{(j)} + aX_{a(j)} + dX_{d(j)} + iX_{i(j)} + LSW_{(j)} * aX_{a(j)} + LSW_{(j)} * dX_{d(j)} + \\ 209 \quad LSW_{(j)} * iX_{i(j)} + e_{(j)} \quad (3)$$

210

211 Models (2) and (3) were then individually compared (using the  $-2$  log likelihood  
 212 values as described above) to a further reduced model (Model 4) that contained only  
 213 the three genetic effects and the two litter size parameters but not the interactions:

214

$$215 \quad Y_j = LSW_{(j)} + LSB_{(j)} + aX_{a(j)} + dX_{d(j)} + iX_{i(j)} + e_{(j)} \quad (4)$$

216

217 Thus, the only difference between Models (2) and (3) is the type of interaction effect  
218 included. Using Models (2), (3) and (4) we generated two tests of interaction effects.  
219 The comparison of Model (2) to Model (4) ( $-2 \log$  likelihood of Model 4 minus that  
220 of Model 2) provides a chi-square test (with 3 d.f.) of the interaction of the three  
221 genetic effects with litter size at birth. The comparison of Model (3) to Model (4) ( $-2$   
222  $\log$  likelihood of Model 4 minus that of Model 3) provides a chi-square test (with 3  
223 d.f.) of the interaction of the three genetic effects with litter size at weaning.  
224 Depending on which of the interaction effects (with litter size at weaning or at birth)  
225 was significant, we identified whether litter size at birth or at weaning is causal to the  
226 interaction effect.

227       To generate significance thresholds we used the effective number of markers  
228 method, which is based on the Eigenvalues of the marker correlation matrix (Li and Ji  
229 2005). This approach calculates the number of independent tests in a genome scan and  
230 adjusts significance using a Bonferroni correction. Briefly, one first calculates the  
231 correlation matrix for the marker loci and then estimates the Eigenvalues of the  
232 correlation matrix. The integer parts of the Eigenvalues are replaced by 1 when the  
233 value is  $\geq 1$  and 0 when the value is  $< 1$ . This integer part is then added to the original  
234 decimal part to yield the effective number of markers contained in that Eigenvalue.  
235 For example, an Eigenvalue of 3.75 yields 1.75 effective markers, while an  
236 Eigenvalue of 0.75 yields 0.75 effective markers. The sum of these converted values  
237 represents the effective number of markers, which we used in the Sidak equation to  
238 generate the threshold for genome-wide tests (i.e. we used the effective number of  
239 markers on the whole genome to generate thresholds). We have previously  
240 demonstrated that the thresholds obtained are very similar to those obtained through  
241 computationally intensive simulation (Hager *et al.*, 2008a). We thus determined the

thresholds for all traits and identified significant loci when the overall locus LPR value or the interaction effect LPR value exceeded the genome-wide threshold. To investigate pleiotropic effects we included QTL effects whenever the effect of a given locus is significant at the pointwise threshold ( $p < 0.05$ ;  $LPR > 1.3$ ) assuming the QTL has exceeded the genome-wide significance threshold for a different trait (Wolf *et al.*, 2008).

We have previously established that parent-of-origin dependent effects on offspring phenotypes may be caused by either maternal genetic effects or genomic imprinting (Hager *et al.*, 2008b). In essence, differences in maternal genotype can cause differences between phenotypes of heterozygous offspring and thus cause the same parent-of-origin effect patterns as those caused by genomic imprinting effects. This also applies to the appearance of additive effects due to the genetic correlation of offspring with their parents at a locus (where, at a particular locus, the correlation is  $\frac{1}{2}$ ). Thus, a locus expressed in the mother may affect her offspring's phenotype, but since offspring inherit one allele from their mother it appears as if that locus directly affects offspring phenotype. This scenario applies to non-cross-fostered animals only as the autocorrelation between maternal and offspring genotype is broken in cross-fostered animals. We therefore tested all loci with a significant interaction to determine whether the interaction effect could be explained by a maternal genetic effect or was associated with a change in the direct effect of a locus. This was achieved by using a mixed model to test whether the parent-of-origin-dependent effect or additive effect differed significantly between individuals reared by homozygous versus heterozygous mothers (Hager *et al.*, 2009a).

## Results

267 We first analyzed main effects of litter size, independent of any genotype effects. The  
268 average litter size at birth in our experimental population was 8.54 with a range from  
269 4 to 13 pups per litter. All traits were highly significantly affected by litter size at  
270 weaning (i.e. post natal litter size), including week 1 body weight, whereas litter size  
271 at birth only affected weeks 1 to 3. Invariably, litter size effects were negative such  
272 that average individual weight decreased as litter size increased. Litter size at birth  
273 had a standardized effect of  $-0.15$  for week 1 weight, decreasing to  $-0.07$  for week 3.  
274 To illustrate the magnitude of these effects we compare litter sizes of five and nine  
275 individuals at week 1. Pups born into the larger litter would then be 14.38% or 0.61 g  
276 smaller compared to those born with five litter mates (average weight at week 1 is  
277 4.23g). Unsurprisingly, the effects of litter size at weaning are greatest for week 2 and  
278 week 3 (NB. cross-fostering took place at birth) with standardized estimates of  $-0.55$   
279 and  $-0.43$ . However, these effects extend all the way to week 10 (standardized  
280 estimate  $-0.19$ ), at which time pups born into litters of nine are still 2.6% smaller on  
281 average compared to those born into litters of five.

282       After having established the main effects of litter size, we performed a genome  
283 scan across all 19 autosomes for loci that showed a significant interaction between  
284 genetic (additive or dominance) or epigenetic (genomic imprinting) effects and litter  
285 size at birth or weaning on weight and growth. We denote loci that show interaction  
286 effects with litter size at birth  $LSBy.z$  and loci interacting with litter size at weaning  
287  $LSWy.z$ .  $LSB$  refers to litter size at birth,  $LSW$  to litter size at weaning,  $y$  identifies the  
288 chromosome and  $z$  the individual QTL on that chromosome in case several QTL are  
289 found on one chromosome. For all loci we confirmed that any imprinting or additive  
290 interaction effect is not caused by a maternal genetic effect.

291 Five loci on separate chromosomes (chromosomes 1, 4, 6, 11 and 16) showed  
292 an interaction with litter size at birth (Table 1). Two loci showed interactions with  
293 additive effects, two loci interacted with dominance and one with imprinting effects.  
294 One might have expected that the interaction of genotype with litter size at birth  
295 predominantly affects early weights, however, late weights are equally affected and  
296 *LSB16.1* only affected body weight from week 5 onwards. Turning to loci that  
297 interacted with litter size at weaning, we identified two QTL located on chromosomes  
298 10 and 15 (Table 1). With the exception of *LSB11.1* all loci affected several traits  
299 showing clearly when during development their effects become manifested, when  
300 they are greatest and when they cease to show detectable effects (see LPR values in  
301 Table 1). A locus on chromosome 10 showed an unusual pattern in that additive  
302 interactions with litter size at birth influenced pre-weaning growth whereas  
303 dominance interactions affected week 4 – 10 (at the same locus).

304

#### 305 *Effects of litter size interactions*

306 The nature of the interaction can be examined by looking at the litter size effect in  
307 individuals with specific alleles at a locus, which can be inferred from the sign of the  
308 interaction given in Table 1. Overall, average body weight decreases with increasing  
309 litter size regardless of genotype at all loci. However, the degree to which increasing  
310 litter size leads to a reduction in weight across genotype varies and is manifested in  
311 the interaction. A positive interaction of additive effects and litter size ( $+a \times LS$ ) means  
312 that *LL* homozygotes show a smaller reduction in weight compared to *SS*  
313 homozygotes with increasing values of the litter size parameter (either litter size at  
314 birth, *LSB*, or litter size at weaning, *LSW*, respectively). The reverse applies to a  
315 negative interaction effect ( $-a \times LS$ ). Positive dominance interactions indicate that

heterozygotes showed a smaller reduction in weight compared to homozygotes with increasing litter size values. Again, the reverse applies to negative dominance interaction effects. Finally, for positive imprinting interactions, *SL* heterozygotes showed a stronger decrease in their weight compared to *LS* heterozygotes with increasing litter size.

Overall, the loci show both positive and negative interactions with litter size for additive and dominance effects. Thus, no general pattern can be established across all traits for the direction of effect for a given genotype such that, for example, *LL* homozygotes always increased in weight with increasing litter size. However, we can illustrate the effects of the interactions for body weight, for example comparing homozygotes and heterozygotes at *LSB16.1* where we assume a difference in litter size of four, e.g. comparing homozygous and heterozygous pups born into litters of five versus those born in litters of nine litter mates (about the average litter size in our population). Figure 1 shows the average phenotype of both homo- and heterozygotes for week 7 bodyweight when litter size at birth was nine next to their average weights when litter size was five. We see that while homozygotes showed a reduction in weight of 11.3%, from their average weight with litter size of nine to their average weight with litter size of five, the corresponding value for heterozygotes was only 4%. The differences between genotypes in their weight increase when comparing litters of greater size difference. For example, focusing on differences between the two heterozygotes (at *LSB6.1*) and comparing litters of five and 11 pups, we find that *SL* heterozygotes would be over 11% smaller while *LS* heterozygotes would be only ~5% smaller in litters of 11 compared to litters of five litter mates.

## Discussion

341 Although the effects of litter size on growth and levels of competition have been  
342 demonstrated in different systems (Mock and Parker 1997; Stockley and Parker  
343 2002), we show that such effects can depend on the genotype of individuals at specific  
344 loci. This advances on previous studies that generally demonstrated a genetic basis for  
345 such interactions (e.g. Merilä and Fry 1998). In addition, our study confirms the  
346 existence of a substantial main effect of litter size on weight such that average weight  
347 was inversely proportional to litter size (e.g. Reading 1966; Epstein 1978). The effects  
348 of litter size on weight decrease over time but even at week 10 are still significant:  
349 body weight at week 10 is affected by litter size at weaning with a standardized effect  
350 of -0.19, which would cause a reduction of 2.6% of weight comparing pups with nine  
351 litter mates at weaning to those that had five litter mates. This suggests that, although  
352 compensation for lower body weight caused by being born in larger litters is possible,  
353 such compensatory growth is only partial and, consequently, a negative effect in terms  
354 of smaller body size remains even weeks later. Our previous work analyzed main  
355 genetic and genomic imprinting effects on weight and growth in this population  
356 (Hager et al. 2009b) and we can thus compare these loci with those found in the  
357 present study. We had discovered 18 main effect loci on 13 chromosomes that show  
358 additive, dominance or imprinting effects (Hager *et al.*, 2009b, Table 1 therein).  
359 While none are identical to the interaction loci we found in this present study, three of  
360 the interaction loci (*LSB4.1*, *LSW10.1* and *LSW15.1*) are located directly adjacent to  
361 main effect loci (*adi4.1*, *adi10.1* and *adi15.1*). The locus on chromosome 4 shows an  
362 additive main effect on weights and growth as well as an interaction of the additive  
363 effect with litter size at birth. Loci on the other chromosomes, however, do not show  
364 the same main and interaction effects and it seems thus less likely that they could be  
365 the same. In conclusion, with one exception on chromosome 4 (*LSB4.1*), the main



366 effect QTL are different from those showing the interaction with litter size suggesting  
367 that the litter size interaction QTL described here are more specifically responding to  
368 the social environment, unlike the main effect QTL,

369         Overall, we detected five loci with effects on weight and growth that were  
370 dependent on (i.e. showed an interaction with) the number of siblings at birth, and two  
371 that were dependent on litter size at weaning. Such genotype by (social) environment  
372 interactions may enable genotypes to respond differentially to changes in  
373 environmental conditions in relation to sibling competition and resource availability.  
374 The differential effects of the prenatal (uterine) competitive environment (i.e. pre-  
375 natal litter size) on postnatal growth could arise from differential genetic priming of  
376 offspring to expectations of future resource availability and sibling competition. For  
377 example, an individual born into a small litter might expect lower competition levels  
378 due to adequate milk supply for all litter mates, whereas individuals born into large  
379 litters may expect high levels of competition. Only a direct investigation of  
380 competitive behaviour of pups cross-fostered into litters that differ in their size at birth  
381 and at weaning would allow a confirmation of this hypothesis.

382         We were able to clearly demonstrate that genetic variants modulate individual  
383 responses to changes in the competitive environment, in essence phenotypic plasticity  
384 in social environments. However, whether or not the observed effects are adaptive is  
385 more difficult to ascertain. If the effects were adaptive and selection favoured larger  
386 body size, then we might expect genotypes adapted to larger litter size to show better  
387 performance (i.e. larger body weight) as litter size increases compared to genotypes  
388 adapted to smaller litter size. However, we do not find a consistent allele effect in  
389 that, for example, individuals being homozygous for the *L* allele show a smaller  
390 reduction in weight (or increased fitness) compared to *SS* homozygotes. Instead, as

391 indicated by the sign of the interaction for additive effects in Table 1, being  
392 homozygous for the *L* allele results in a negative effect at one locus (*LSB1.1*), but a  
393 positive effect at another (*LSB4.1*). For example, one might have expected that in the  
394 strain that produces larger litters (*Large* has an average litter size of 6.1 compared to  
395 an average litter size of *Small* of 5.0, a difference of 18%; Ehrich *et al.*, 2003), levels  
396 of competition are higher than in strains with smaller litters and thus *Large* pups were  
397 to some degree selected to develop in a more competitive environment. The *L* alleles  
398 might thus confer a competitive advantage compared to *S*, reflected in increased  
399 fitness of *LL* homozygotes when litters increase in size. This is not the case. Similarly  
400 inconsistent is the pattern of interaction for dominance interactions, which overall  
401 suggests non-adaptive reasons for the observed plasticity.

402         One explanation for the absence of clear adaptive benefits of plasticity is that  
403 the loci may have pleiotropic effects on other traits that are not plastic (DeWitt *et al.*,  
404 1998). While this remains an untested possibility, it seems more likely that the  
405 condition of litter size change experienced in our experimental population is outside  
406 the range of conditions in the original populations (Ghalambor *et al.*, 2007). Given  
407 that the average litter size and range in our population exceeds that in both pure  
408 strains by about three individuals, levels of competition may be different as may be  
409 the costs (Chappel *et al.*, 2002).

410         The key result emerging from our study is that a given genotype results in  
411 different phenotypes depending on the number of siblings. This genotype by social  
412 environment interaction can be similar in magnitude as the strong main effects of litter  
413 size (which results in considerable reduction in weight with increasing litter size) and  
414 thus should thus be ascribed biological importance equivalent to the main effect. In  
415 contrast to litter size main effects, the genotype by litter size interactions do not show

416 a consistent effect across genotypes. Therefore, although genetic variation to respond  
417 to changes in the competitive environment exists, the observed phenotypic plasticity  
418 in our study may be regarded as non-adaptive at present.

419

420

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#### 428 **Conflict of interest**

429 The authors declare no conflict of interest.

430

431

#### 432 **Data archiving**

433 Data identifiers to be added

434

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526 **Figure legends**

527

528 **Figure 1:** Difference in average week 7 bodyweight for the homozygotes and  
529 heterozygotes at *LSB16.1* shown for two different litter sizes at birth. The graph  
530 illustrates that homozygotes suffer a greater reduction in weight than heterozygotes  
531 for the same litter size difference. Error bars indicate the standard error of the mean.  
532



**Table 1.** Interaction QTL with litter size at birth (*LSB*) and litter size at weaning (*LSW*). The first column identifies the QTL followed by the genomic location in F<sub>2</sub> equivalent centiMorgans (cM) and the coordinates in megabases (Mb) based on mouse genome build 36. This is followed by the traits affected. The column ‘Full LPR’ lists the overall model LPR for the test of main effects and interactions effects. Under ‘Interaction effect’ we specify which of the three effects showed an interaction with the sign of effect, where ‘*a*’ refers to additive, ‘*d*’ to dominance and ‘*i*’ to imprinting effects. In parentheses we give the interaction estimates. The column headed ‘Test’ gives the LPR for the interaction effect. ‘Growth 13’ and ‘Growth 310’ refer to pre-weaning and post-weaning growth, respectively. Confidence intervals (Mb) for the interaction QTL positions were determined using a one LOD drop (using LPR values) following Lander & Botstein (1989). Because a locus may affect several traits, the LPR for the interaction effects may be different and hence the confidence intervals.

QTL	Location		Confidence	Trait	Full LPR	Interaction effect	Test
	cM	Mb	interval			(estimate)	
<b><i>LSB1.1</i></b>	42.66	93.21	87,02 – 99,36	Week 1	4.18	<i>a</i> (0.036)	1.58
			87,02 – 99,36	Week 2	6.85	<i>a</i> (0.063)	2.98
			87,02 – 99,36	Week 3	3.30	<i>a</i> (0.078)	1.98
<b><i>LSB4.1</i></b>	37.28	84.31	78,87 – 87,93	Week 4	5.11	<i>-a</i> (-0.161)	2.43
				Week 5	5.02	<i>-a</i> (-0.172)	1.85
				Week 6	6.06	<i>-a</i> (-0.195)	1.72
<b><i>LSB6.1</i></b>	29.25	65.37	105,62 – 117,19	Week 4	7.79	<i>-i</i> (-0.131)	1.69
			94,76 – 134,72	Week 5	7.44	<i>-i</i> (-0.181)	1.99
			94,76 – 134,72	Week 6	7.21	<i>-i</i> (-0.242)	2.43
			94,76 – 134,72	Week 7	6.76	<i>-i</i> (-0.302)	2.82
			94,76 – 134,72	Week 8	6.50	<i>-i</i> (-0.320)	2.64
			94,76 – 134,72	Week 9	6.97	<i>-i</i> (-0.406)	3.50
			94,76 – 134,72	Week 10	5.68	<i>-i</i> (-0.384)	2.86
			94,76 – 134,72	Growth 3-10	4.99	<i>-i</i> (-0.003.)	2.44
<b><i>LSW10.1</i></b>	49.16	111.3	99,22 – 117,08	Week 4	2.66	<i>-d</i> (-0.223)	2.62
			99,22 – 128,85	Week 5	4.59	<i>-d</i> (-0.265)	2.41
			99,22 – 117,08	Week 6	3.86	<i>-d</i> (-0.263)	1.79

			99,22 – 128,85	Week 7	3.97	<i>-d (-0.295)</i>	1.73
			99,22 – 117,08	Week 8	4.88	<i>-d (-0.370)</i>	2.12
			99,22 – 117,08	Week 9	4.68	<i>-d (-0.383)</i>	1.99
			99,22 – 117,08	Week 10	4.72	<i>-d (-0.443)</i>	2.30
<b>LSB11.1</b>	11.90	24.47	60,48 – 72,28	Week 4	2.37	<i>d (0.243)</i>	3.14
<b>LSW15.1</b>	30.19	69.47	62,91 – 76,75	Week 2	3.49	<i>d (0.074)</i>	2.71
			56,81 – 83,18	Week 3	4.63	<i>d (0.127)</i>	3.15
			62,91 – 76,75	Week 4	2.16	<i>d (0.162)</i>	1.66
<b>LSB16.1</b>	44.61	46.92	36,99 – 50,54	Week 5	2.16	<i>d (-0.336)</i>	3.73
			36,99 – 50,54	Week 6	2.84	<i>d (-0.454)</i>	4.64
			36,99 – 50,54	Week 7	3.32	<i>d (-0.559)</i>	5.27
			36,99 – 50,54	Week 8	2.90	<i>d (-0.613)</i>	5.14
			36,99 – 50,54	Week 9	2.72	<i>d (-0.624)</i>	4.70
			36,99 – 50,54	Week 10	2.95	<i>d (-0.713)</i>	5.36
			36,99 – 50,54	Growth 310	3.81	<i>d (-0.714)</i>	6.10

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